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Concentration of Poliovirus in Water by Molecular Filtration

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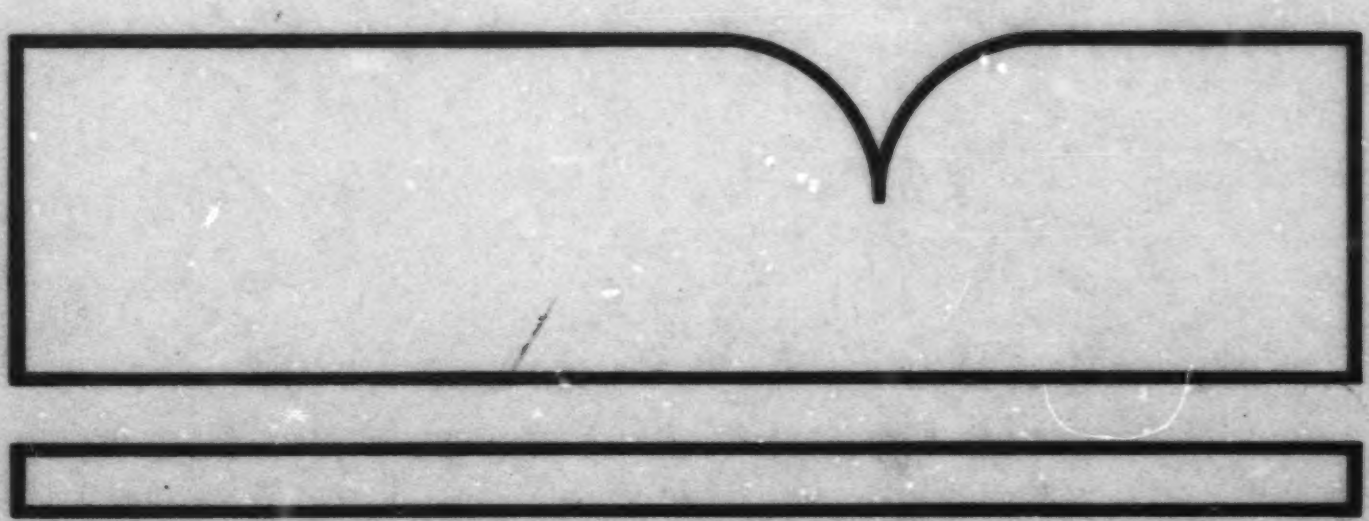

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Concentration of Poliovirus in Water by Molecular Filtration

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The efficiency of concentrating poliovirus 1 from distilled water samples was determined by using a recirculating-flow molecular filtration system. The most efficient recoveries were achieved against membranes with a 10,000 nominal molecular weight limit pretreated with flocculated beef extract. This procedure yielded a mean virus recovery of 67%.

Judicious selection of a quantitative method for concentrating viruses to a point at which they can be reliably assayed is essential in monitoring for viruses in water. Recent changes in molecular filtration equipment and membranes have provided an innovative approach to virus concentration and have been effectively applied in the concentration of laboratory-cultured viruses (4, 6). Molecular filtration has a particular advantage over procedures currently used to concentrate waterborne viruses (1, 3, 8, 9, 11) because it is not dependent on virus adsorption to a matrix and consequently minimizes virus loss resulting from competition with other organic matter for adsorption sites. Some concentration procedures also require pH changes and polycation additions to obtain more efficient virus adsorption. On the other hand, concentration by molecular filtration is not based on the net charge of the virus particle, thus eliminating the need for acidifying or adding polycation salts.

The molecular filtration unit (Pellicon cassette system, Millipore Corp., Bedford, Mass.) was equipped with two PT series membranes which had a total surface area of 920 cm^2 and a nominal molecular weight limit of either 10,000 or 100,000. Two of the four ports connected to the manifold of the unit served as filtrate outlets. The sample-retentate flow, to and from the unit, was through the remaining two ports. Sterilization was achieved by recirculating a 0.1% solution of NaOCl for 10 min. This was followed by a sterile water wash which was continued until water leaving all ports was free of chlorine by the *o*-tolidine spot test. Virus feed samples at a final concentration of 1 to 10 plaque-forming units per ml were prepared by rapidly mixing poliovirus 1 (Mahoney LP) in 2- or 4-liter volumes of distilled water. Flow of these samples through the cassette system was regulated so that separation was achieved at a retentate-to-filtrate flow ratio of 1:1. The retentate was recirculated until only 1 to 2 ml of fluid remained in the dispensing vessel. After clamping off both filtrate lines and inserting the retentate outlet tubing into a receiving vessel, the remaining 1 or 2 ml of fluid followed by air was pumped through the unit to force the total retentate volume from the cassette and tubing. The filtrate and retentate volumes were recorded, and a portion of each was assayed. Virus assays were carried out with the BGM cell line as described previously (2).

Initially, membranes with a 100,000 nominal molecular weight limit were used. Table 1 shows that virus recoveries in this system after 23- to 46-fold concentrations averaged only 10.5% and that less than 1% of the unaccounted-for virus was present in the filtrate. In an attempt to increase recoveries, both water and beef extract were passed through the cassette as a forward wash. Neither method resulted in virus recoveries of better than 10%. Backwashing the membranes with water also gave very limited recoveries. To test for the presence of toxic products, distilled water was passed through the membrane and tubing, inoculated with virus, and titrated. No toxic effects were observed.

To check the possibility that the virus was nonreversibly adsorbed on the membranes, we made single runs in which 1% glycine was used either to pretreat the membrane or to supplement the feed. Either treatment resulted in the apparent blockage of virus adsorption sites. When combined, the average virus recovery increased to 39.0% (Table 2). Although crude beef extract clogged the membranes, beef extract flocculated by an acid precipitation procedure (5) and readjusted to pH 7 could be used to pretreat the membrane. By pretreatment with flocculated beef extract, a nearly fivefold increase in virus recovery was observed. At the same time the number of virions passing into the filtrate averaged 20.4% of the total viral input. Such virus recoveries from the filtrate were deemed unacceptable and led to the use of membranes with a 10,000 nominal molecular

TABLE 1. *Concentration of poliovirus 1 from distilled water by molecular filtration against membranes with a 100,000 nominal molecular weight limit*

Vol filtered (liters)	Virus recovery (%)	
	Retentate	Filtrate
2	14.3	<1
	13.1	<1
	10.4	<1
	5.7	<1
	3.4	<1
4	15.9	<1

TABLE 2. *Effect of pretreating membranes (100,000 nominal molecular weight limit) and suspending medium on concentration of poliovirus 1 by molecular filtration*[a]

Pretreatment of membrane[b]	Suspending medium	Virus recovery (%)	
		Retentate	Filtrate
Glycine (1%)	Water	24.3	8.9
None	Glycine (1%)	25.8	3.3
Glycine (1%)	Glycine (1%)	50.6	<1
		39.3	
		38.4	
		27.8	4.3
Flocculated 3% beef extract[c]	Water	75.4	24.1
		66.0	19.4
		54.4	10.1
		45.9	12.2
		33.3	36.0

[a] No appreciable differences noted in efficiency of recovery when 2- or 4-liter volumes were filtered.

[b] Membranes pretreated by recirculating a 500-ml volume through the Pellicon cassette system at a retentate-to-filtrate flow ratio of 1:1, with residual fluid retained in the cassette.

[c] Beef extract flocculated by acid precipitation (5) and the aqueous portion readjusted to pH 7.

weight limit. As shown in Table 3, virus suspended in distilled water and concentrated against these membranes without pretreatment again resulted in low (17%) virus recoveries. Feed supplement, membrane pretreatment, or a combination thereof with 1% glycine also increased virus recovery significantly, as did membrane pretreatment with flocculated 3% beef extract. The passage of virus through membranes with a 10,000 nominal molecular weight limit was negligible and may have accounted for the increased virus recoveries in the retentate.

Pretreatment of the membranes with flocculated 3% beef extract yielded a substantially

TABLE 3. *Effect of pretreating membranes (10,000 nominal molecular weight limit) and suspending medium on concentration of poliovirus 1 by molecular filtration*[a]

Pretreatment of membrane[b]	Suspending medium	Virus recovery (%)	
		Retentate	Filtrate
None	Water	17.8	1
		16.9	<1
	Glycine (1%)	32.5	<1
		29.9	<1
Glycine (1%)	Water	42.7	<1
	Glycine (1%)	56.7	<1
		43.6	<1
Flocculated 3% beef extract[c]	Water	76.8	<1
		73.4	<1
		49.6	1.4

[a] Two-liter volumes filtered.

[b] Membranes pretreated by recirculating a 500-ml volume through the Pellicon cassette system at a retentate-to-filtrate flow ratio of 1:1, with residual fluid retained in the cassette.

[c] Beef extract flocculated by acid precipitation (5) and the aqueous portion readjusted to pH 7.

higher average virus recovery (66.6%) than did membrane pretreatment with 1% glycine alone (42.7%) or in combination with 1% glycine feed supplementation (50.2%). This difference in recoveries at the near-neutral pH range at which samples were processed might have been anticipated by comparing these results with those from earlier studies (7, 10) with glycine or beef extract as eluents for recovery of adsorbed viruses. Even after flocculation, beef extract has been highly effective for the release of such viruses (unpublished data).

The results of these tests indicate that the described molecular filtration system can effectively concentrate viruses from water, provided membranes are pretreated with flocculated beef extract. However, clarification may be required for water containing suspended particles larger than 10 μm. Since samples can be concentrated to about 100 ml or less, this system can also be used as a second-step concentration procedure when the volume of primary concentrate must be further reduced before virus assay.

LITERATURE CITED

1. Berg, G., D. R. Dahling, and D. Berman. 1971. Recovery of small quantities of viruses from clean waters on cellulose nitrate membrane filters. Appl. Microbiol. 22: 608–614.

2. **Dahling, D. R., G. Berg, and D. Berman.** 1974. BGM, a continuous cell line more sensitive than primary Rhesus and African Green kidney cells for the recovery of viruses from water. Health Lab. Sci. 11:275–282.
3. **Farrah, S. R., S. M. Goyal, C. P. Gerba, C. Wallis, and J. L. Melnick.** 1978. Concentration of poliovirus from tap water onto membrane filters with aluminum chloride at ambient pH levels. Appl. Environ. Microbiol. 35:624–626.
4. **Gangemi, J. D., E. V. Connell, B. G. Mahlandt, and G. A. Eddy.** 1977. Arenavirus concentration by molecular filtration. Appl. Environ. Microbiol. 34:330–332.
5. **Katzenelson, E., B. Fattal, and T. Hostovesky.** 1976. Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. Appl. Environ. Microbiol. 32:638–639.
6. **Mathes, L. E., D. S. Yohn, and R. G. Olsen.** 1977. Purification of infectious feline leukemia virus from large volumes of tissue culture fluids. J. Clin. Microbiol. 5:372–374.
7. **Rao, V. C., U. Chandorkar, N. U. Rao, P. Kumaran, and S. B. Lakhe.** 1972. A simple method for concentrating and detecting viruses in wastewater. Water Res. 6:1565–1576.
8. **Sobsey, M. D., and B. L. Jones.** 1979. Concentration of poliovirus from tap water using positively charged microporous filters. Appl. Environ. Microbiol. 37:588–595.
9. **Sobsey, M. D., C. Wallis, M. Henderson, and J. L. Melnick.** 1973. Concentration of enteroviruses from large volumes of water. Appl. Microbiol. 26:529–534.
10. **Wallis, C., M. Henderson, and J. L. Melnick.** 1972. Enterovirus concentration on cellulose membranes. Appl. Microbiol. 23:476–480.
11. **Wallis, C., A. Homma, and J. L. Melnick.** 1972. A portable virus concentrator for testing water in the field. Water Res. 6:1249–1256.